The Roles of Depot Injection Sites and Proximal Lymph Nodes in the Presystemic Absorption of Fluphenazine Decanoate and Fluphenazine: *Ex Vivo* Experiments in Rats

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Purpose. The release and presystemic absorption of fluphenazine and its decanoate ester from intramuscular depots were investigated.

Methods. Rats were sacrificed in groups of three at various times after injection of drug or prodrug in sesame oil. Muscle tissues at the injection sites and various lymph nodes were excised. Blood (plasma) was harvested by cardiac puncture.

Results. Following administration of fluphenazine decanoate, the amount of prodrug at the sites of injection declined exponentially (half-life 3.4 days). Highest concentrations of drug and prodrug were found in iliac and hypogastric lymph nodes nearest to injection sites in which both analytes were detectable 28 days post dose. The half-life for the decline of fluphenazine from lymph nodes (4.6 days) was similar to that from plasma (4.3 days). Following administration of fluphenazine base, only 2.8% of the dose remained at the sites of injection after 2 days. Concentrations of drug in iliac and hypogastric lymph nodes were comparable to those in distal lymph nodes. Fuphenazine concentrations in the lymphatic tissues decreased at about the same rate as plasma concentrations.

Conclusions. The rate limiting step appeared to be slow partitioning of the decanoate from oily deposits at the injection site and proximal lymph nodes with subsequent hydrolysis of the ester group.

KEY WORDS: fluphenazine decanoate; prodrug; fluphenazine; lymph nodes; intramuscular administration.

INTRODUCTION

Fluphenazine decanoate is an ester of fluphenazine and decanoic acid that is widely used in the maintenance treatment of schizophrenia and other psychotic disorders. It is formulated as an oil based intramuscular "depot" injection with which patients are typically injected once every 2–3 weeks (1,2). Nevertheless, many questions still remain concerning the pharmacokinetic properties of the prodrug. In particular, there were no data on the fate of the intact ester in the body until recent reports of the detection of fluphenazine decanoate in the plasma of schizophrenic patients under maintenance therapy with the prodrug (3) and in the plasma of dogs following single intramuscular doses of fluphenazine decanoate in sesame oil (4). Moreover, there is little information on the mechanisms and the route(s) of absorption of the prodrug from oily depots. One study in dogs with ^{14}C-fluphenazine decanoate, found that about 18.6 % of the dose remained at the injection site 35 days after dosing (5). Those data should be interpreted cautiously, however, in view of the use of non-specific total radioactivity measurements. In a more recent study (4), dramatic differences were observed in the kinetic profiles of fluphenazine following intramuscular administration of sesame oil solutions of drug or prodrug which suggested differences in the rate limiting presystemic absorptive processes. Experiments with radio-labeled oil containing no prodrug revealed the presence of oil in lymph nodes (6) of experimental animals. Radio-labeled haloperidol decanoate and to a lesser extent, haloperidol were detected in lymph nodes from various parts of the bodies of rats after 50 mg/kg of ^{14}C-haloperidol decanoate was given intramuscularly in sesame oil, which suggested that haloperidol decanoate is at least partially absorbed via the lymphatic system (7). More recent studies (8) demonstrated that concentrations of fluphenazine in the lymph of rats were comparable to those in plasma, suggesting that the lymphatic system plays a role in the absorption of fluphenazine after intramuscular administration of its decanoate ester in sesame oil. The present study was carried out to investigate the processes involved in the presystemic absorption of both fluphenazine and its decanoate ester after injection.

MATERIALS AND METHODS

Materials

Fluphenazine decanoate was kindly supplied by Bristol Myers Squibb (Montreal, Canada) while fluphenazine dihydrochloride was purchased from Sigma-Aldrich Canada Ltd. All other chemicals and solvents were of analytical grade and were used without further purification.

Animals and Drug Treatment

Sprague-Dawley female rats weighing about 300 g were used in all experiments which were carried out according to the Principles of Laboratory Animal Care (NIH Publication #85-23, revised 1985) and under conditions specified by the Canadian Council on Animal Care. In the first experiment, 21 rats were injected intramuscularly with fluphenazine decanoate (10 mg/kg) dissolved in sesame oil (25 mg/ml) at 2 sites of each femoral muscle (a total of 4 injections). Groups of 3 rats were sacrificed at 1, 2, 4, 7, 14, 21, 28 days post dose. In the second experiment, 9 rats were injected intramuscularly with fluphenazine free base (10 mg/kg) dissolved in sesame oil (25 mg/ml) at 2 sites of each femoral muscle (a total of 4 injections). Groups of 3 rats were sacrificed at 0.5, 1, 2 days post dose. Control rats were sacrificed similarly after intramuscular injections of sesame oil.

Blood and Tissue Sampling

Blood was collected by cardiac puncture from rats under ether anesthesia and was centrifuged to give plasma. After the latter procedure, the rats were sacrificed by further ether inhalation. Muscle tissues around the injection sites and lymph

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nodes from various parts of the body were quickly removed, weighed and homogenized with 0.1 N HCl to halt enzyme activity. All samples were stored at −20°C until analyzed.

Analytical Procedure

Fluphenazine decanoate and/or fluphenazine concentrations in plasma and tissue homogenates were determined using a validated HPLC method based on a published procedure (3). Quality control samples were prepared by adding known amounts of fluphenazine decanoate or fluphenazine to blank plasma or blank tissue homogenates and analyzing these samples blindly at the same time as the test samples to establish sample stability and assay accuracy and precision. The drug(s) and the internal standard (perphenazine) were extracted from samples and chromatographed under isocratic conditions. Quantitation was achieved using the analyte/internal standard peak height ratio.

RESULTS AND DISCUSSION

Disappearance of Fluphenazine Decanoate and Fluphenazine from Intramuscular Oily Depots

The percentages of the dose of ester remaining at the injection sites as fluphenazine or its decanoate after intramuscular administration of prodrug in sesame oil are shown in Figure 1. The prodrug exhibited exponential disappearance characteristics such that half of the dose of fluphenazine decanoate disappeared after about 3.4 days and 0.42% of the decanoate ester was still present at the injection sites on day 28 postdose. Initial concentrations of fluphenazine at the sites of injection were considerably lower than those of the prodrug which suggests that ester hydrolysis occurred to a limited extent in the muscle tissue (Figure 1). Earlier work (8) showed that fluphenazine decanoate is hydrolyzed relatively rapidly in rat muscle tissue homogenates. In the present study, however, the prodrug is not available for hydrolysis until after the ester partitions out of the sesame oil. The latter appears to be the rate limiting step. Unchanged ester was also carried with the oil from the depot sites to iliac and hypogastric lymph nodes close to the injection sites. These data suggest that the exponential disappearance

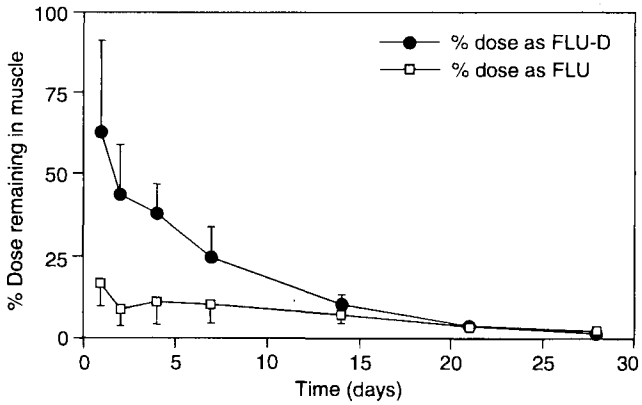

Fig. 1. Mean (±SD, n = 3) percentage of dose remaining at the injection sites as fluphenazine decanoate (dark circles) and fluphenazine (light squares) after intramuscular administration of fluphenazine decanoate in sesame oil.

of fluphenazine decanoate from the injection sites is in part dependent on the slow partitioning of the prodrug out of the oil and subsequent relatively rapid hydrolysis by the muscle esterases and in part by absorption of the intact prodrug into the lymph nodes. When fluphenazine base was injected in sesame oil, however, the rate of disappearance of the drug from the muscle tissue was much more rapid than that observed after administration of the prodrug such that only 2.8% of the dose of fluphenazine remained at the sites of injection two days post dose. This indicates that fluphenazine partitions from the sesame oil into the interstitial aqueous phase much more rapidly than does the decanoate ester.

Levels in Lymph Nodes

An earlier study (8) found that concentrations of fluphenazine in rat lymph (thoracic duct) were comparable with those in plasma after intramuscular administration of the decanoate in sesame oil. The present *ex vivo* study focused on concentrations of drug and prodrug in lymph nodes rather than the lymph *per se*. Of the lymph nodes examined, highest concentrations of drug and prodrug were found in iliac and hypogastric lymph nodes after intramuscular administration of fluphenazine decanoate in sesame oil. These lymph nodes are positioned on the lower dorsal abdominal wall close to the injection sites. The same phenomenon has been observed previously after intramuscular administration of haloperidol decanoate in sesame oil (9). Figure 2A provides a dramatic illustration of high concentrations of both fluphenazine and its decanoate ester in these proximal lymph nodes, whereas comparatively minuscule concentrations of fluphenazine only were found in more distal lymph nodes and in the plasma. In fact plasma concentrations were 216 times lower than those in proximal lymph nodes at 1 day post dose, and decreasing progressively to 1560 times lower at 21 days post dose. Moreover, both drug and prodrug were present in the proximal lymph nodes 28 days post dose when neither was detectable in the distal lymph nodes or in the plasma. The latter are illustrated in Figure 2B in which the expanded y-axis shows that concentrations of fluphenazine in plasma in the same order as those in the superior mesenteric and neck peripheral lymph nodes. These data suggest that most of the decanoate ester and some of the fluphenazine in the proximal lymph nodes was mostly derived from the injection sites. The lymph sites in the neck and mesenteric areas, however, have no direct connection with the proximal lymph nodes and it would appear likely that fluphenazine in the distal lymph nodes was derived from the blood stream. Thus it was confirmed that the local lymphatic system is involved in the presystemic absorption of the decanoate from intramuscular depot sites in rats.

Fluphenazine base in sesame oil was administered intramuscularly to investigate the consequence of the lack of an ester group. Once again, highest concentrations of the drug were found in the proximal lymph nodes although the levels were far lower than those found in these tissues after administration of the prodrug (12 fold lower at day 1) as illustrated in Figure 3 which is drawn on the same scale as Figure 2A for comparative purposes. This implies a somewhat smaller portion of the dose was carried with the oil into the lymphatic system after administration of fluphenazine base. Moreover, it is also apparent that in the absence of prodrug, no sustained lymphatic

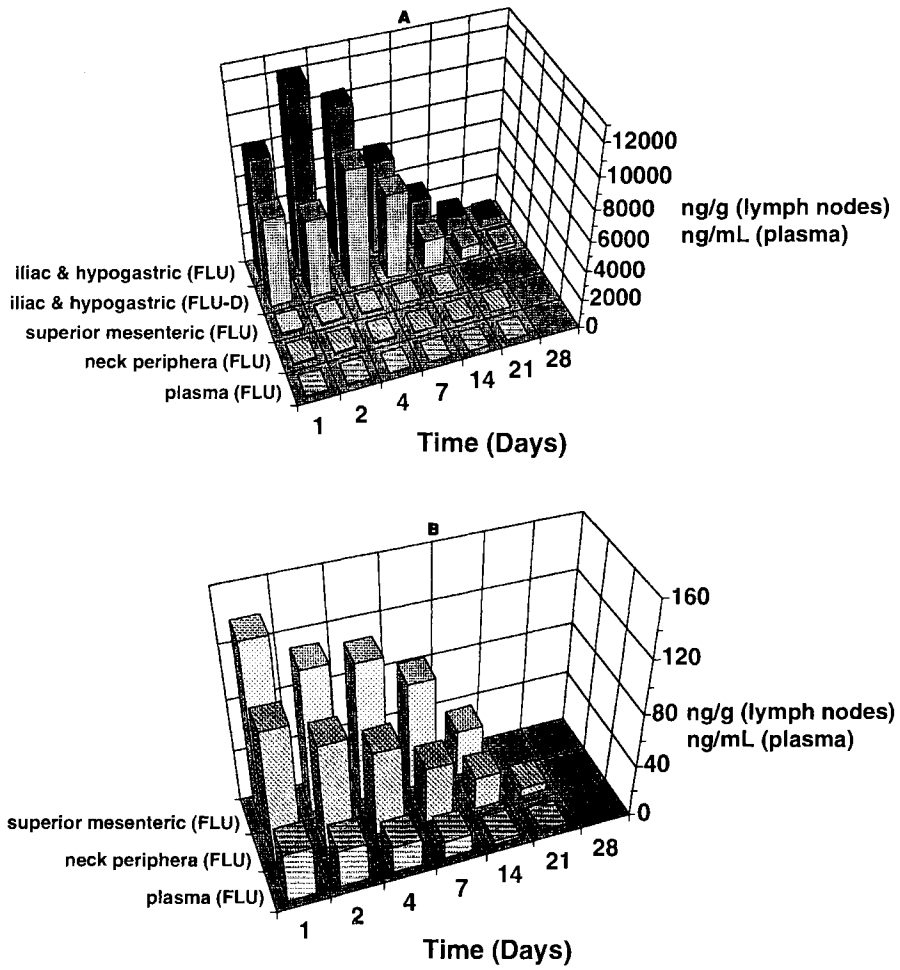

Fig. 2. *Ex vivo* concentrations of fluphenazine decanoate and fluphenazine in various lymph nodes and in plasma after intramuscular administration (rat femoral muscle) of fluphenazine decanoate in sesame oil. 2B. Same as 2A with 75 fold expanded of the y-axis to show concentrations of fluphenazine in the distal lymph nodes and in plasma.

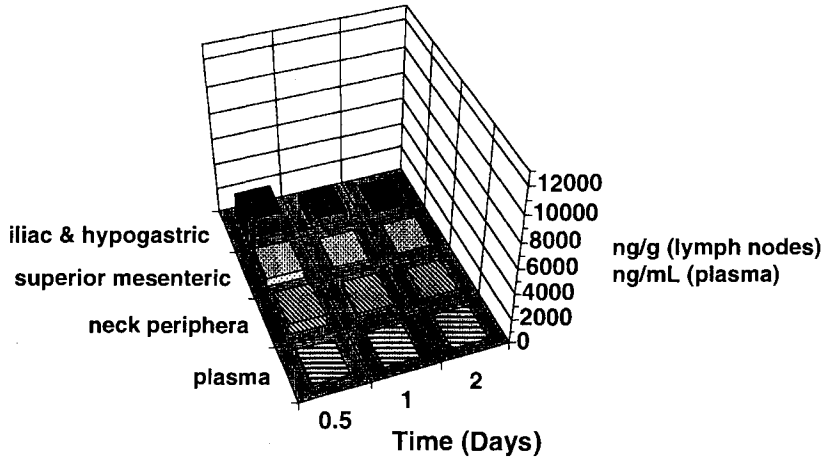

Fig. 3. *Ex vivo* concentrations of fluphenazine decanoate and fluphenazine in various lymph nodes and in plasma after intramuscular administration (rat femoral muscle) of fluphenazine base in sesame oil. 2B.

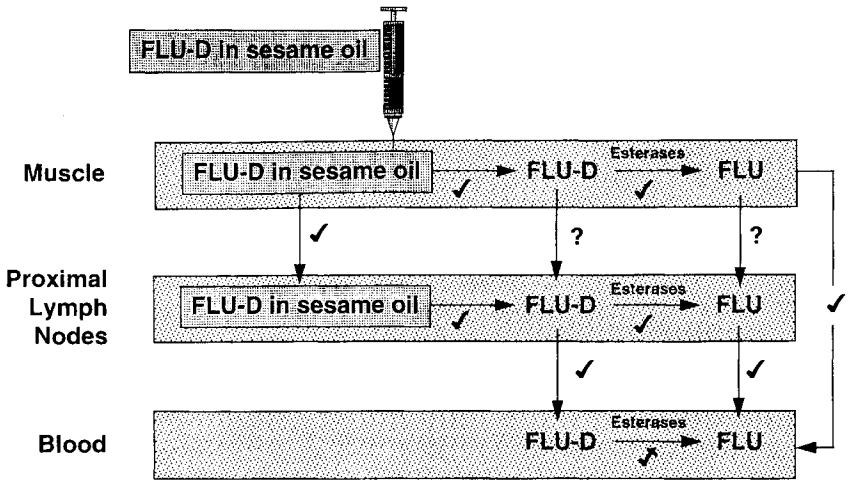

Fig. 4. Putative mechanisms and route(s) of absorption of fluphenazine and fluphenazine decanoate after intramuscular injection of the prodrug in sesame oil into the femoral muscles of rats. The check marks indicate processes for which there is evidence. The crossed check mark indicates a process rapid in rat but much slower in dog and human.

uptake of fluphenazine base was observed since concentrations in plasma and all lymphatic tissues examined declined with comparative rapidity such that no drug was detectable in any tissue beyond two days post dose. These data suggest that the sesame oil carried a portion of the fluphenazine base into the proximal lymph nodes where the drug partitioned out of the oil more rapidly than the highly lipophilic prodrug, while fluphenazine that partitioned out of the oil at the injection site was probably taken up directly into the bloodstream. After administration of fluphenazine decanoate in sesame oil, the sustained high levels of fluphenazine in the lymph nodes (Figure 2A) appeared to have arisen mostly from hydrolysis of the decanoate in situ rather than by direct uptake of fluphenazine from the injection sites. Earlier work (7,9) has shown that lymphocytes trapped in the lymph nodes possess hydrolytic activity for ester prodrugs. The rate limiting step in the presystemic absorption of both fluphenazine and the decanoate, however, appears to be the slow partitioning of drug/prodrug out of the sesame oil at the injection sites and in the proximal lymph nodes.

Plasma Concentrations

In the present experiments, no unchanged fluphenazine decanoate was detected in plasma 1 day after intramuscular administration of the prodrug to rats although in pilot experiments, low concentrations of fluphenazine decanoate was detected within 12 hours of dosing in some rats. The dearth of prodrug in rat plasma is probably due to the relatively high hydrolytic capacity of rat plasma esterase which have been shown to possess greater hydrolytic capacities than those in dog or human plasma (8,10). After intramuscular administration of the decanoate, the half life for decline of plasma fluphenazine (4.3) days resembled that of the rate of decline from the proximal lymph nodes (4.6 days) which is somewhat longer than the disappearance half-life of prodrug from the injection sites (3.4 days). This suggested that the relatively long half life of fluphenazine in rat plasma was a function of a slow rate of input governed by the retention of the prodrug in oil at the injection site and in proximal lymph nodes.

Plasma levels of fluphenazine were not sustained after intramuscular administration of fluphenazine base in sesame oil. After administration of the prodrug in sesame oil, however, sustained plasma levels of the fluphenazine indicate that the ester group plays a very important role by conferring greater retention in the oil as a consequence of the greater lipophilicity of the prodrug. These observations are consistent with an *in vivo* study (4) in dogs that showed the clearance and volume of distribution of fluphenazine after intramuscular administration in sesame oil (10.9 L/h, 118.8 L) were roughly double corresponding values (5.8 L/h, 51.0 L) after intravenous fluphenazine whereas half lives were comparable (im 7.7, iv 6.0 h). The half life of fluphenazine after intramuscular administration of the decanoate in sesame oil, however, was more than 30 fold greater (233 h).

CONCLUSIONS

The foregoing data on the absorption of intramuscularly injected fluphenazine decanoate in sesame oil are summarized conveniently in Figure 4. After intramuscular administration of fluphenazine decanoate in sesame oil to rats, slow hydrolysis of the prodrug by muscle esterases and absorption of the oily solution of the prodrug into the proximal lymph nodes are mechanisms that both contribute to the exponential disappearance of the ester from the injection sites. Therefore plasma fluphenazine could be derived primarily from the hydrolysis of the ester in both of these sites and subsequently in other tissues including blood. The latter possibility is shown in Figure 4 with a crossed check mark because this route is fast in rat but slow in dog and human. The rate limiting step in the absorption or release kinetics of fluphenazine and its prodrug could be the slow partitioning of prodrug from oily deposits at the injection site and in the proximal lymph nodes with subsequent hydrolysis of the ester groups.

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